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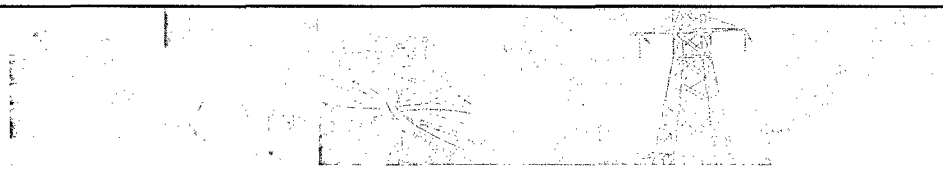

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Pharmacokinetic Studies of Intramuscular Midazolam in Guinea Pigs Challenged with Soman[#]

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ABSTRACT

Studies have demonstrated that benzodiazepine compounds are effective at antagonizing seizure activity produced by the organophosphate (OP) cholinesterase inhibitor soman. In this present study we have investigated the pharmacokinetics of midazolam and its associated effects on electroencephalographic (EEG) activity following intramuscular (im) injection to soman-exposed guinea pigs (Crl:(HA)BR). Prior to experiments, the animals were surgically implanted with EEG leads to monitor seizure activity. For the study, animals were administered the following pretreatment/OP/treatment regimen. Pyridostigmine bromide (0.026 mg/kg, im) was given 30 min prior to soman (56 μg/kg, 2 × LD_{50}; subcutaneously, sc), followed in one minute by atropine sulfate (2 mg/kg, im) and pralidoxime chloride (25 mg/kg, im). All animals receiving this regimen developed seizure activity. Midazolam 0.8 mg/kg, im, was administered 5 min after onset of seizure activity. Based on EEG data, animals were categorized as either seizure-terminated or seizure not-terminated at 30 min following anticonvulsant administration. Serial blood samples were collected

[#]In conducting the research described in this report, the investigators complied with the regulations and standards of the Animal Welfare Act and adhered to the principles of the Guide for the Care and Use of Laboratory Animals (NRC 1996).

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for the plasma midazolam analysis; the assay was accomplished with a gas chromatograph/mass spectrometer. The mean time to seizure termination was 8.8 ± 1.6 min. The mean time-plasma concentration data were fit to standard pharmacokinetic models. The following parameter estimates were determined from the model-fit for seizure terminated and not-terminated animals respectively: apparent volumes of distribution (Vd) were 1.4 and 1.7 l/kg; area under the time-concentration curves (AUC), 15,990 and 15,120 ng · min/ml; times to maximal plasma concentration (T_{max}), 1.66 and 2.91 min and maximal plasma concentrations (C_{max}) 535.1 and 436.6 ng/ml. These data indicate that im injection of midazolam is effective at terminating ongoing soman-induced seizure activity. Additionally, the relatively short T_{max} and latency to seizure termination demonstrate the rapidity of drug absorption and action respectively.

Key Words: Intramuscular; Midazolam; Benzodiazepine; Anticonvulsant(s).

INTRODUCTION

Intoxications with organophosphate (OP) cholinesterase inhibitors, such as soman, produce a progression of toxic signs, including hypersecretions, motor convulsions, seizures, and death (Taylor, 1996). Irreversible inhibition of acetylcholinesterase (AChE) results in cholinergic over-activity as a consequence of the acetylcholine accumulation. Research has demonstrated that OP-induced lethality can be reduced utilizing a carbamate cholinesterase inhibitor (pretreatment) followed by atropine and oxime treatment postexposure (Berry and Davies, 1970; Dirnhuber et al., 1979; Fleisher and Harris, 1965; Gordon et al., 1978; Leadbeater et al., 1985). As a result, a combined regimen of prophylaxis (i.e., pretreatment) and postexposure therapy has been suggested to be the most effective countermeasure for intoxication (Dunn and Sidell, 1989; Sidell, 1992). Pyridostigmine (PYR) pretreatment is employed in military operations where potential exposure to nerve agents exists. Its role is to reversibly inhibit the active site of AChE, thereby protecting it from irreversible phosphorylation by the OP. Protection of AChE is based upon the spontaneous decarbamylation of PYR-inhibited enzyme. Atropine antagonizes excess acetylcholine at muscarinic receptors while 2-PAM can potentially displace the OP from the inhibitor-enzyme complex and reactivate the free enzyme. However, the combination does not address the development of seizure activity, progression to status epilepticus and associated neuropathology induced by OP exposure. For optimal patient outcome, the addition of an anticonvulsant is necessary. Diazepam has been suggested as the drug of choice for the treatment of OP poisoning (Vale and Scott, 1974). It has been shown to prevent the onset and to greatly reduce the intensity of soman-induced seizure/convulsions (Lipp, 1972, 1973) as well as neuropathology (Hayward et al., 1990). It is currently included with the standard therapeutic regimen for OP nerve agent intoxication and provided as a 10 mg auto-injector to be utilized for intramuscular (im) self-administration. Although the efficacy of intravenous (iv) diazepam in treating status epilepticus in a clinical emergency is well documented, its usefulness in a military setting is less clear. The issue centers about the im route of administration necessitated by the autoinjector delivery system, and its impact on achieving efficacious plasma levels with a relatively short latency to drug action. Data extrapolated from pharmacokinetic studies in soman-exposed guinea pigs

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suggest that im administration of 3 autoinjectors of diazepam (30 mg) to a 70–75 kg human (0.4–0.43 mg/kg) may result in less than optimal plasma levels and latency to drug action in an emergency military treatment setting (Capacio et al., 2001).

Midazolam has been shown to be effective for treating seizure/convulsions associated with exposure to multiple lethal doses of OP cholinesterase inhibitors (Anderson et al., 1997; Shih et al., 1991). Additionally, in guinea pigs with soman-induced seizure activity, midazolam was shown to act more quickly and at much lower doses than diazepam following im administration (McDonough et al., 1999). The objective of these studies was to investigate the pharmacokinetics and tissue distribution of midazolam after im administration while monitoring pharmacodynamic (electroencephalographic; EEG) data in soman-exposed guinea pigs.

METHODS

Animals

Male Hartley Crl: (HA)BR COBS® guinea pigs weighing 250–300 g, obtained from Charles River Laboratories (Wilmington, MA), were utilized for all experiments. All animals were maintained under an Association for the Assessment and Accreditation of Laboratory Animal Care—International (AAALAC-I) program and housed individually in polycarbonate cages. They were provided commercial certified guinea pig ration as appropriate and tap water ad libitum. The guinea pig holding room was maintained at 21 ± 2°C with 50 ± 10% relative humidity. The holding room was ventilated (at least 10–15 complete changes per hour) with 100% conditioned fresh air and was maintained on a 12-hour light/dark full spectrum lighting cycle with no twilight.

Chemicals

All chemicals and solvents were of HPLC grade or higher. Pyridostigmine, atropine sulfate, pralidoxime chloride, and midazolam were obtained as dry powders from the Department of Experimental Therapeutics, Walter Reed Army Institute of Research. Desmethyldiazepam for use as internal standard was obtained as a dry powder from Sigma Chemical Co. (St. Louis, MO). Soman (pinacolyl methylphosphonofluoridate) was obtained from the U. S. Army Edgewood Chemical Biological Center (Aberdeen Proving Ground, MD, USA). All pretreatment/treatment compounds and midazolam for intramuscular (im) administration to guinea pigs were dissolved in normal saline.

Surgery

Animals were prepared for stainless cortical screw electrodes according to previously described procedures (McDonough and Shih, 1993). For anesthesia, animals were induced in a plexiglas chamber with 3% isoflurane and quickly transferred to a

stereotaxic frame where they were maintained to effect with 1–2% via mask. Approximately 7–10 days for recovery were allowed prior to experimentation.

Experimental Procedures

Electroencephalographic activity was monitored and recorded continuously during the entire experiment where guinea pigs received the following OP pretreatment/ treatment drug regimen: PYR (0.026 mg/kg; im) 30 min prior to soman (2 × LD_{50}, 56 μg/kg; subcutaneously, sc) followed in one minute by atropine sulfate (2 mg/kg, im) and 2-PAM (25 mg/kg; im). Midazolam was given 5 min after onset of electrographic seizures. To obtain plasma and tissue concentration-time data, animals were divided into groups according to tissue collection times (30, 60, 120, 180 and 240 min after midazolam). Serial blood samples were taken from all animals at 0, 1, 8 and 15 min. Depending on sacrifice time, samples were also obtained at 30, 60, 120, 180 and 240 min after midazolam; the last blood sample was obtained immediately prior to tissue collection. This procedure resulted in five experimental groups with serial blood samples as well as central (brain stem, cerebellum, cortex) and peripheral (diaphragm, kidney, liver, skeletal muscle) tissues collected at the termination of the experiment. Seizure was categorized as either seizure ON or seizure OFF based on the individual EEG signal at 30 min after midazolam administration. If during the experimental period an animal ceased seizure activity beyond 30 min following midazolam, that time to termination was noted but was included in the seizure ON category.

Midazolam Assay

Sample Preparation

Whole blood samples (500 μl) were obtained from a toenail clip procedure utilized previously (Capacio et al., 1997) and collected in microfuge tubes treated with heparinized saline as an anticoagulant. Tissues were homogenized in a volume of normal saline equivalent to two times their weight. All samples (whole blood or tissue homogenates) were centrifuged for 20 minutes (2000 × g) at 5°C; then 200 μl of either plasma or supernatants were collected. To all samples (200 μl), desmethyldiazepam (5 μl, 5 μg/ml) was added as an internal standard. The samples were extracted with borate buffer (75 μ, pH = 7) and methylene chloride (2.0 μm). The organic layer was removed and placed in glass vials where it was evaporated under a gentle stream of nitrogen at room temperature. Subsequently they were reconstituted with 100 μl ethylacetate and analyzed by GC-MS under the conditions described.

Gas Chromatography-Mass Spectrometry (GCMS)

The midazolam GCMS assay was carried out according to the methods describes by Byers et al. (2001). Gas chromatographic separations were performed on an Agilent 6890 gas chromatograph system. The GC was fitted with a 30 m × 0.25 mm I.D. DB-5MS bonded phase column, 0.25 μm film thickness (J&W Scientific, Folsom, CA). Helium was used as the carrier gas at a constant pressure of 14.9 psi. The oven temperature was held initially at 120°C for 1 min, ramped from 120 to 240°C at

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42°C min^{-1}, and held at 240°C for 0.5 min; then ramped to 280°C at 15°C min^{-1} and held 4 min, then finally ramped to 310°C at 30°C min^{-1} for 0.5 min. Splitless injections of 1 µl volume were made using an Agilent 7683 autosampler.

Electron ionization MS analyses were performed on an Agilent 5973 mass selective detector interfaced to the GC above. The electron ionization MS operating conditions were as follows: ion source pressure approximately 1.5×10^{-5} torr; source temperature, 230°C; electron energy, 70 eV; and electron emission current, 50 uA. The mass spectrometer was operated using selected ion monitoring (SIM). Two ions characteristic for each desmethyldiazepam (*m/z* 235 and 272) and midazolam (*m/z* 310 and 324) were monitored at a dwell time of 50 ms each. This resulted in a total scan rate of 7.41 cycles sec^{-1}.

Electroencephalographic Recording

Electroencephalographic recordings were made using QND software and amplifiers supplied by the Neurodata Inc. (Pasadena, CA) (low-frequency filter = 0.3 Hz; high-frequency filter = 40 Hz; sampling rate = 128 Hz) and displayed on a computer monitor. During EEG recordings, all animals were housed in individual plastic recording chambers that allowed free movement with the exception of the recording leads attached to the connector on top of the head.

Pharmacokinetic Analysis

Mean plasma time-concentration data were fit to standard pharmacokinetic models using WinNonlin (version 1.5, 1997, Scientific Consulting, Inc. Cary, NC) non-linear regression software. The analysis of mean plasma concentration-time data generated observed versus predicted concentrations as a function of time as well as pharmacokinetic parameter estimates. The choice of the appropriate model was based upon the best fit of the raw data to the mathematical model. The following criteria were utilized as guidelines for determining the appropriate model: minimal sum of squared residuals, high correlation coefficient, small standard deviations of parameter estimates and unbiased distribution patterns of residuals for estimates of observed versus predicted values. The analysis of data generated pharmacokinetic parameter estimates from mean raw data for seizure-terminated and seizure not-terminated groups. Parameter estimates generated were apparent volume of distribution (Vd), absorption rate constant (k_{01}), elimination rate constant (k_{10}), time to maximum plasma concentration (T_{max}), maximum plasma concentration (C_{max}), half-lives associated with absorption and elimination ($T_{1/2}$-abs and $T_{1/2}$-elim) respectively, and area under the time-concentration curve (AUC).

Statistical Analysis

Statistical calculations were accomplished with GraphPad Prism version 3.02 for Windows (GraphPad Software, San Diego, California, 2000). Plasma and tissue concentrations for seizure ON vs seizure OFF groups were compared at each time point using a one-way analysis of variance (ANOVA). To identify significant differences

Table 1. Midazolam dose-related incidence of seizure termination.

Midazolam dose (mg/kg, im)	Response fraction[a]	Percent terminated
0.5	0/5	0
0.75	2/7	28
0.8	3/7	43

[a]Number of animals (per total) in which seizures were terminated within 30 min of midazolam administration.

between ON and OFF at a given time for each plasma and each tissue, a Bonferroni's multiple comparison test was run. Significance was considered at a level of $p < 0.05$. Brain (brain stem, cerebellum, cortex) and peripheral tissue (kidney, liver, diaphragm, skeletal muscle) concentrations were compared with plasma concentrations at each time point using a paired t-test. Significance was considered at a level of $p < 0.01$.

RESULTS

Termination of Seizure Activity

The model utilized in this study induced seizures in 100% of the animals. In a preliminary dose ranging study, midazolam, 0.8 mg/kg, terminated soman-induced seizure activity in 43% (response fraction = 3/7) of the animals examined (Table 1). Because this dose provided an approximately equal split (i.e., number terminated vs not terminated) in the animal population, it was utilized in the full study. In the full study, the overall seizure termination rate within 30 min was 38% across all time points (Table 2). Of the animals in which seizures were not controlled within 30 min, only one animal in the 60-min time group stopped seizing before the end of the experiment (42.0 min). The mean time to termination was 8.8 min within the 30-min time window and 10.3 min for all animals in which seizure activity was terminated regardless of the time constraint. The remainder (60%) experienced either persistent or intermittent seizure activity through the end of the study (Table 2).

Table 2. Seizure termination in time study groups.

	Experiment duration (min)					Overall
Category	30 (n = 10)	60 (n = 15)	120 (n = 10)	180 (n = 10)	240 (n = 10)	(n = 55)
% OFF before 30 min	30	40	40	30	50	38
Mean time (min)	16.1	13.2	4.2	5.5	4.5	8.8
% OFF after 30 min	0	7	0	0	0	2
Mean time (min)	N/A	42.0	N/A	N/A	N/A	42.0
% ON at end of experiment	70	53	60	70	50	60

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Table 3. Midazolam pharmacokinetic parameter estimates.

Parameter	Pharmacokintic parameter estimate (± SE)	
	Seizure OFF	Seizure ON
Vd (l/kg)	1.4 (0.05)	1.67 (0.06)
AUC ng · min/ml	15,990 (688)	15,120 (654)
$T^1/_2$-abs (min)	0.26 (0.05)	0.53 (0.05)
$T^1/_2$-elim (min)	19.53 (1.32)	21.89 (1.42)
T_{max} (min)	1.66 (0.25)	2.91 (0.18)
C_{max} (ng/ml)	535.08 (14.07)	436.60 (11.06)

Pharmacokinetics and Tissue Distribution

The pharmacokinetics of midazolam after im administration to guinea pigs were best described by a one-compartment model with first-order absorption and elimination described by Eq. 1 (below).

$$C(t) = \frac{D}{Vd}\frac{k_{01}}{k_{01} - k_{10}}(e^{-k10t} - e^{-k01t}) \quad (1)$$

Where C = plasma concentration (ng/ml), t = time (min), D = dose (μg/kg), Vd (l/kg), k_{01} (minutes^{-1}), and k_{10} (minutes^{-1}). The pharmacokinetic parameter estimates were generated for animals categorized as seizure OFF and seizure ON using mean plasma concentration-time data. The pharmacokinetic parameter estimates for both groups are presented in Table 3.

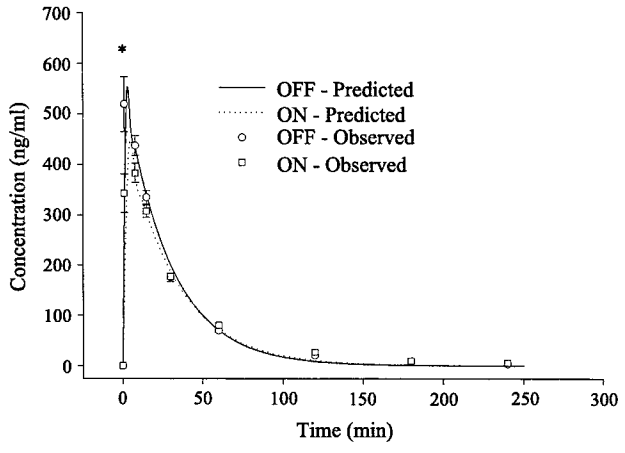

Figure 1. Observed and Predicted Midazolam Concentrations in Guinea Pigs Categorized as Seizure OFF or Seizure ON as a Function of Time: One compartment model predicted pharmacokinetic curves for guinea pigs classified as seizure OFF (solid line) or seizure ON (dotted line) are presented along with observed mean plasma concentrations (OFF, circle; ON, square). *Denotes significant difference in concentrations between OFF and ON groups at corresponding time points.

Across time, plasma concentrations for the seizure OFF group tended to be higher than those for seizure ON; however, only at the 1-min time point was the difference in concentration found to be significant ($p < 0.05$). The model-predicted plasma concentrations along with mean observed concentrations for seizure OFF and seizure ON groups are presented graphically in Figure 1.

Midazolam concentrations in regional brain tissue (cortex, brain stem and cerebellum) from 30 through 240 min are presented along with overall mean plasma concentration-time data in Figure 2A. Maximum concentrations were observed at the first time point (30 min) for all three-brain areas. Concentrations in all the brain areas were similar at each time point. Midazolam plasma concentrations were significantly

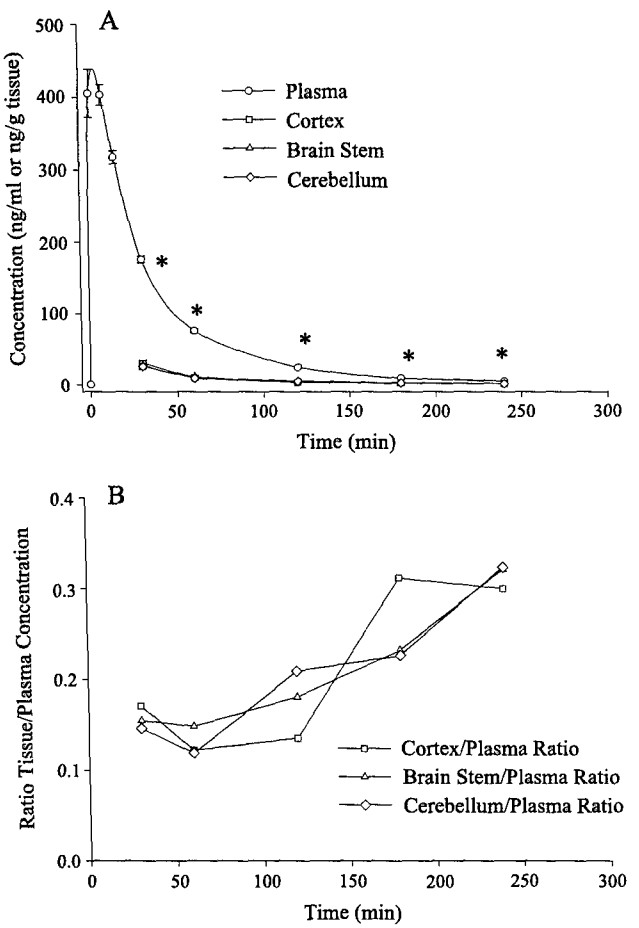

Figure 2. (A) Time-Course of Midazolam in Regional Brain Areas and Plasma: Concentrations of midazolam in cortex, brain stem and cerebellum (ng/g tissue) are presented along with mean concentration-time profile of midazolam in plasma (ng/ml). (B) Ratio Brain Area/Plasma Concentration: The ratio of cortex, brain stem and cerebellum to plasma concentration are shown. *Denotes significant difference between concentration in plasma and three-brain areas at corresponding time points.

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greater than those in each brain area at corresponding time points ($p < 0.01$). The ratio of brain area/plasma midazolam concentrations ranged from 0.14 at 30 min to 0.32 at 240 min (Figure 2B). Overall, the brain area to plasma concentration ratios demonstrated an increasing trend with time.

Midazolam concentrations in kidney and liver tissue from 30 through 240 min are presented along with overall mean plasma concentration–time data in Figure 3A. Similar to the brain area data, maximal midazolam concentrations were detected at the initial time point (30 min). Liver concentrations were found to be statistically greater ($p < 0.01$) than plasma concentrations from 60 through 240 min. The ratios of liver/plasma concentrations increased with time from 1.5 at 30 min to 4.0 at 240 min

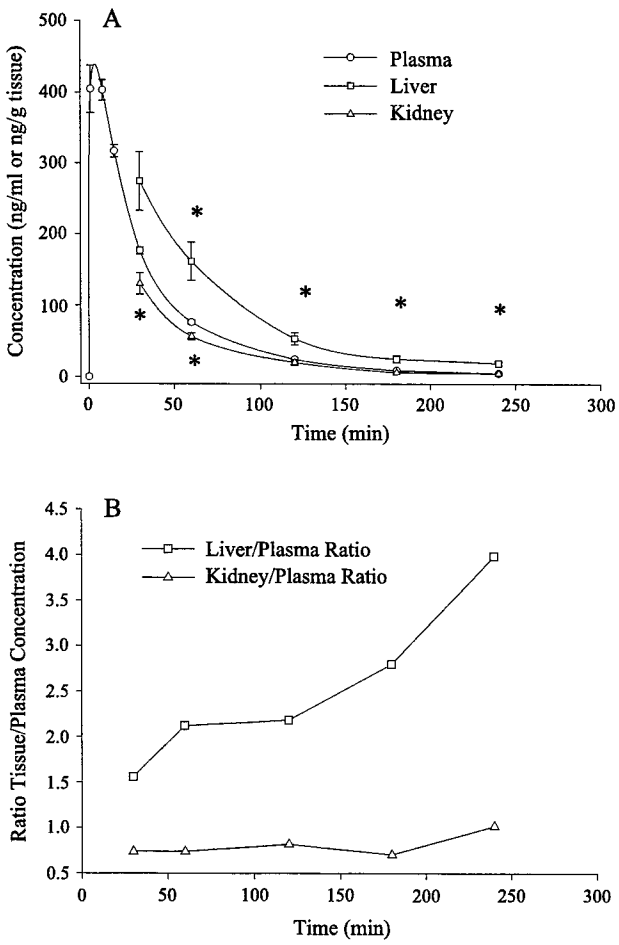

Figure 3. (A) Time-Course of Midazolam in Liver, Kidney and Plasma: Concentrations of midazolam in kidney and liver (ng/g tissue) are presented along with mean concentration-time profile of midazolam in plasma (ng/ml). (B) Ratio Liver, Kidney/Plasma Concentration: The ratio of liver and kidney to plasma concentration are shown. *Denotes significant difference between tissue concentration and plasma at corresponding time points.

(Figure 3B). The concentration of midazolam in plasma was significantly greater than in kidney at the 30- and 60-min time points. The ratio of kidney/plasma concentrations ranged from 0.74 to 0.82 at 30 through 180 min (Figure 3B). At 240 min the kidney/plasma ratio was 1.0. Whereas the brain areas and liver to plasma ratios increased with time, the kidney to plasma ratio remained fairly constant over time.

Similar to the time-course profiles observed in the brain areas, kidney and liver, maximum midazolam concentrations were observed at the 30-min time point in skeletal muscle and diaphragm. For skeletal muscle, midazolam concentrations at 30, 60, 120 and 180 min were statistically less ($p < 0.01$) than those in plasma at corresponding time points (Figure 4A). The skeletal muscle/plasma concentration ratios ranged from

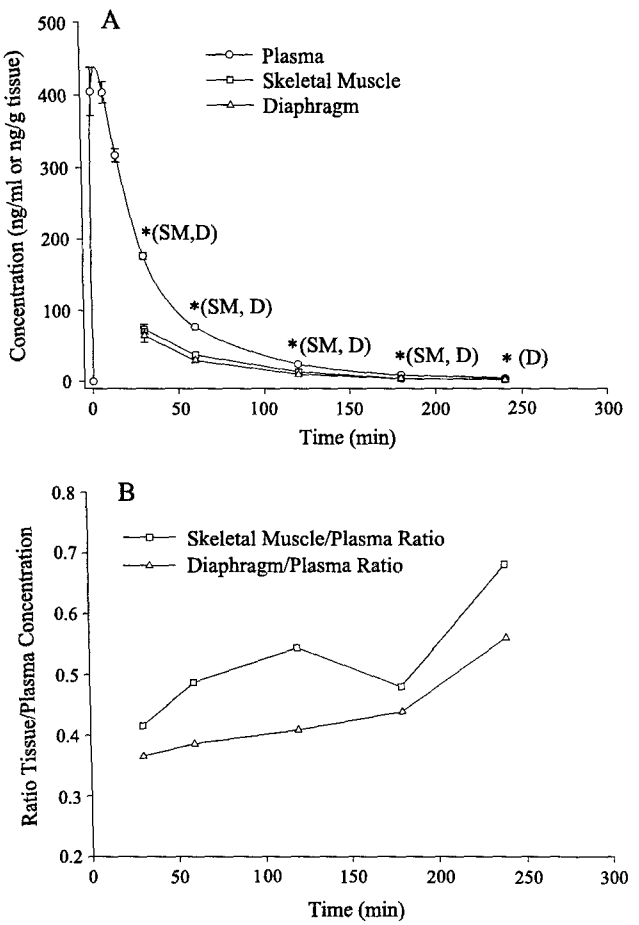

Figure 4. (A) Time Course of Midazolam in Skeletal Muscle, Diaphragm and Plasma: Concentrations of midazolam in skeletal muscle and diaphragm (ng/g tissue) are presented along with mean concentration–time profile of midazolam in plasma (ng/ml). (B) Ratio Skeletal Muscle, Diaphragm/Plasma Concentration: The ratio of skeletal muscle and diaphragm to plasma concentration are shown. *Denotes significant difference between tissue (SM-skeletal muscle, D-diaphragm) and plasma concentrations at corresponding time points.

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0.41 at 30 min to 0.68 at 240 min (Figure 4B). The diaphragm had statistically less ($p < 0.01$) midazolam concentrations than plasma at all time points studied. The diaphragm/plasma concentration ratios ranged from 0.36 at 30 min to 0.56 at 240 min (Figure 4B). Skeletal muscle and diaphragm/plasma ratios demonstrated an increasing trend with time.

DISCUSSION

The focus of this study was to examine the pharmacokinetics and the pharmacodynamics of midazolam in PYR-pretreated, soman-challenged, atropine/2-PAM-treated guinea pigs. The pharmacokinetics of midazolam in soman-intoxicated guinea pigs were characterized by a one-compartment model with first-order absorption and elimination as described mathematically in Eq. 1. Plasma pharmacokinetic parameter estimates (Table 2) as generated from the non-linear regression program were similar for seizure OFF and seizure ON animals. Comparison of mean raw plasma concentrations between seizure OFF and seizure ON populations demonstrated, in general, greater concentrations in the OFF group. However, only at the 1-min time point were the differences in the mean plasma concentrations statistically significant ($p < 0.05$). Also, for brain area and peripheral tissue concentrations there were no statistically significant differences between seizure ON and seizure OFF animals.

Since animals were treated with anticonvulsant 5 min after onset of seizures, we investigated specifically the ability of im midazolam to terminate ongoing soman-induced seizures. When EEG activity was assessed at 30 min following the administration of midazolam, 38% (mean time = 8.8 min) of the animal population had no evidence of seizure activity and remained seizure free for the duration of the experiment (Table 2). Beyond the 30-min time window, one animal (2%) had seizure termination that occurred at 42 min. The remainder of the animals experienced either continuous or intermittent seizure activity through the duration of the experiment. The latency to seizure termination (8.8 min) in our study is in the same range as that determined by McDonough et al. (1999), who reported a mean latency time of 13.4 min for midazolam in a similar animal exposure model. These EEG observations demonstrate that a rapid drug action on inhibiting seizures is most likely to occur in the first 30 min after administration.

Midazolam was rapidly absorbed, demonstrating a T_{max} (mean seizure ON and seizure OFF) of 2.3 min. Additionally, decreases in spike amplitude of the EEG pattern were noted within 30 sec to 1 min following administration. By comparison, im administration of diazepam in similar studies resulted in a T_{max} value of 17.5 min (Figure 5) (Capacio et al., 2001). The rapid absorption of midazolam compared with diazepam has been attributed to its relatively greater water solubility. This is in contrast to diazepam, which displays incomplete and erratic im absorption (Hillestad et al., 1974; Towne and DeLorenzo, 1999). The difference in speed of absorption is manifested as a more rapid control of seizure activity. The latency to termination of soman-induced seizure activity for midazolam and diazepam has been studied. McDonough et al. (1999) indicated that the latency to seizure termination was 51.16 and 13.43 min respectively for diazepam and midazolam. Capacio et al. (2001) noted that the mean overall time for diazepam to terminate soman-induced seizures in guinea

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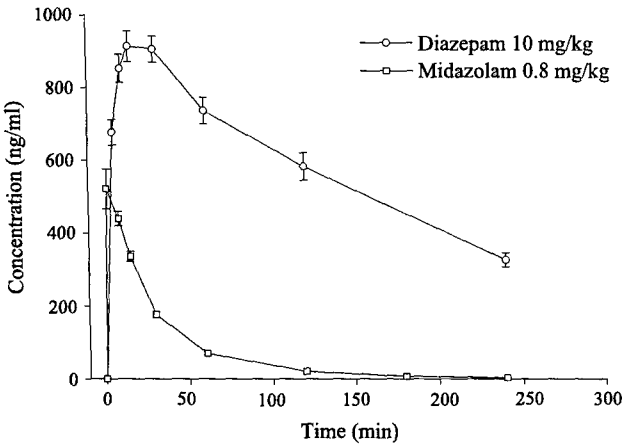

Figure 5. Time Course of Diazepam 10 mg/kg and Midazolam 0.8 mg/kg Following Intramuscular Administration to Soman-Intoxicated Guinea Pigs: Diazepam data from Capacio et al. (2001).

pigs was 39.5 min. By comparison, the overall time to seizure control for midazolam in our studies was 10.3 min and is reflective of the relatively short T_{max} of 2.3 min. The rapidity of absorption as demonstrated by a relatively short latency to seizure termination and T_{max} is critical, regardless of seizure etiology, to preventing serious sequelae and improving prognosis (Towne and DeLorenzo, 1999).

In these studies we have noted relatively small values for Vd (1.5 l/kg) when compared with other anticonvulsants such as diazepam and biperiden studied in this laboratory in a similar model (Capacio et al., 2001, 2003). This apparent volume of distribution reflects the limited distribution into both central and peripheral tissues compared with those compounds. Data from these current studies indicate that the concentrations of midazolam in all tissues, except the liver, were less than those found in plasma. Midazolam concentrations in the brain areas were significantly less (14–32%, $p < 0.01$) than those found in plasma at all time points observed. Midazolam concentrations in the skeletal muscle (except at 240 min) and diaphragm were also significantly less (36 to 56%, $p < 0.01$) than those in plasma at corresponding times. The concentration of midazolam in the kidney relative to that in the plasma was significantly less at the 30- and 60-min time points (74%, $p < 0.01$) and approached 100% at later time points. The liver was the only tissue examined to demonstrate significantly greater midazolam concentrations than those found in plasma. Concentrations of midazolam in the liver relative to plasma increased with time and ranged from 155% at 30 min to 398% at 240 min. This observation is consistent with the studies that indicate the drug is eliminated almost exclusively by metabolic processes in the liver in rats (Woo et al., 1981) and humans (Dundee et al., 1984; Gerecke, 1983; Greenblatt and Abernethy, 1985; Heizman and Ziegler, 1981) The distribution data are in sharp contrast to that determined for the anticonvulsant biperiden determined under similar conditions (Capacio et al., 2003). In those studies biperiden demonstrated extensive distribution (Vd = 14.2 l/kg). Concentrations in brain areas were found to be

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1.4- to 2.2-fold that in plasma, whereas in peripheral tissues, concentrations were as much as eight-fold that in plasma. Studies with diazepam following im administration of 10 mg/kg im resulted in a Vd of 10.1 l/kg. The value is almost 10-fold that determined for midazolam in this study (Capacio et al., 2001). The difference in the Vd's can be explained in part by the depot formation of diazepam in the muscle after im administration (Raines et al., 1990) as well as by a higher brain area to plasma ratio of diazepam (20–60%) relative to midazolam (14–32%).

The $T_{1/2}$-elim of midazolam in this study was 20.7 min. The elimination is approximately 7-fold faster than that demonstrated by diazepam (147.6 min) after im administration to soman-intoxicated guinea pigs (Capacio et al., 2001). The rapid elimination relative to diazepam and other benzodiazepines has also been noted in humans (Reves et al., 1985). The shorter half-life has been reported to be due to a more rapid clearance of midazolam 5.8–9.0 ml/min/kg relative to diazepam and accounts for its reportedly short duration of action (Nordt and Clark, 1997; Reves et al., 1985). Additionally, a faster fall in plasma concentration relative to the diaphragm, liver and skeletal muscle during the course of the experiment is demonstrated by the observation that the respective tissue/plasma concentration ratio increased over time.

Initial studies by Egli and Albani (1981) demonstrated the rapid onset of action and efficacy of im midazolam for terminating seizures in adult humans. Following the administration of 0.15–0.3 mg/kg those studies reported 80% seizure termination. Changes in EEG were shown to start 30 sec to 5 min after injection. Jawad et al. (1986) indicated 15 mg (0.2 mg/kg) of midazolam to be equally effective as diazepam 20 mg (0.26 mg/kg) iv. The efficacy of im midazolam against seizures in humans has also been reported by others (Galdames et al., 1997; Ghilain et al., 1988; Wroblewski and Joseph, 1992). Overall, the literature indicates that studies focused on the utility of im midazolam in adult humans have employed doses in the range of 0.13 to 0.3 mg/kg with efficacies from 80–100%. Following im administration, peak midazolam concentrations have been reported/summarized in humans (Holazo et al., 1988; Hung et al., 1996; Kanto, 1985) to be in the range of 110 to 168 ng/ml when normalized to a dose of 0.1 mg/kg. Therefore, it follows that the administration of 0.3 mg/kg, the upper level of doses used for human seizures, would be expected to result in roughly 3-fold the plasma levels (i.e., 330–504 ng/ml). The plasma levels are consistent with the C_{max} values (436–535 ng/ml) obtained in our studies following im administration to seizing guinea pigs. These data suggest that plasma concentrations from our study, which terminated soman-induced seizures, are in the range of plasma levels that would be expected following im administration of effective doses in clinical epilepsy studies, and therefore, may be predictive of efficacy against nerve agent-induced seizures in humans.

In summary, we have studied the pharmacokinetics and pharmacodynamics of im midazolam in soman-intoxicated seizing guinea pigs. Midazolam exhibited a rapid absorption and elimination profile. At a dose of 0.8 mg/kg ongoing seizures were terminated in 38% of the animals within 30 min of administration. The rapid absorption (T_{max} of 2.3 min) was reflected in the mean time to seizure termination (8.8 min). Plasma levels determined in this study as useful in terminating nerve agent-induced seizures are consistent with those predicted following effective im midazolam dosing in human seizure disorders.

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